Supplemental Material

Materials and Methods

Rationale for calculating Hazard Quotients

Although the effects of POPs on the health of free ranging loggerhead sea turtles (*Caretta caretta*) has been well investigated (Keller et al. 2004; Keller et al. 2006a; Keller et al. 2006b), there was only one previous study on the toxicological effects of POPs on sea turtle embryonic development (Podreka et al. 1998). However, there are a number of studies on freshwater turtles that have investigated the effects of POPs on embryonic sex reversal. Based on these studies, the PNECs could be determined for *p,p'*-DDE, dieldrin and PCBs. The PNEC for *p,p'*-DDE was estimated as 543 ng g^{-1}, based on a study on *C. mydas* that found no sex reversal of developing embryos up to a concentration of 543 ng g^{-1} (Podreka et al. 1998). A study on red-eared slider turtles (*Trachemys scripta*) found no significant sex reversal when embryos were dosed with 5 μL of 2.6 μM dieldrin (Willingham and Crews 1999). Based on an estimated penetration of 30% and an average egg mass of ~ 10 g, this dose represented egg concentration of ~ 40 ng g^{-1} dieldrin. Therefore, after applying an uncertainty factor of 10 for inter-species differences, the PNECs for dieldrin were estimated at 4 ng g^{-1}. The effects of PCBs on sex reversal have also been studied on *T. scripta* (Bergeron et al. 1994). In this study, the topical administration of 10 μg of single PCB compounds to *T. scripta* eggs produced no sex reversal. Based on an estimated penetration of 30% and an average egg mass of 11.4 g, these doses represented egg concentrations of ~ 260 ng g^{-1}. Therefore, after applying an uncertainty factor of 10 for inter-species differences, the PNEC for a single PCB was 26 ng g^{-1}.

Information on the toxicological effects of heavy metals on sea turtles is limited to a single study on the effects of mercury on the immune function of free ranging juvenile loggerhead turtles, *Caretta caretta* (Day et al. 2007). Studies on the toxicological effects of heavy metals have generally focussed on invertebrates and fish and are scarce for oviparous reptiles and birds. However, due to the profound difference in ovipary of these species, the estimation of PNECs for *C. mydas* from invertebrate and fish studies was not considered valid. The PNECs in the present study were therefore limited to the toxic metals and selenium that were estimated from reptile and bird toxicological studies. The PNEC for lead was calculated from the no observed adverse effect level (NOAEL) determined for *T. scripta* by Burger et al. (1998). An uncertainty factor of 100 was applied to the NOAEL value of 100 $\mu g\ g^{-1}$ (x 10 for inter-species differences, x 10 for the use of a sub-chronic NOAEL). A PNEC of 1 $\mu g\ g^{-1}$ was therefore estimated for lead. There was no toxicological information on oviparous reptiles for selenium, cadmium or arsenic. The PNECs for selenium were therefore estimated as 0.34 and 6 $\mu g\ g^{-1}$, based on assumptions and calculations from bird egg studies detailed in Lam et al. (2006). The PNEC for cadmium was estimated from the NOAELs of two dosing studies on broiler chickens. In studies by Voleda et al. (1997) and Leach et al. (1979), egg cadmium concentrations of 1.3 $\mu g\ g^{-1}$ and 0.14 $\mu g\ g^{-1}$, respectively, had no influence on embryo mortality or eggshell thinning. An uncertainty factor of 100 was applied to each of these NOAELs to account for inter-species differences and the use of sub-chronic exposure. This resulted in lower and upper estimates for the PNEC for cadmium to be 0.0014 and 0.013 $\mu g\ g^{-1}$, respectively. The PNEC for arsenic was estimated from a feeding study on mallard hens (Stanley et al. 1994). There were no observable adverse effects on duckling production, mortality or hatch success in eggs with arsenic concentrations of 1.8

μg g^{-1}. Therefore, after the uncertainty factor of 100 was applied to this NOAEL, the PNEC for arsenic was estimated at 0.018 μg g^{-1}.

Results

Supplemental Material, Figure 1. Locations of markets in Peninsular Malaysia selling *Chelonia mydas* eggs for human consumption (top) and the nesting locations from where the eggs were collected (bottom).

References

Bergeron JM, Crews D, McLachlan JA. 1994. PCBs as environmental estrogens: Turtle sex determination as a biomarker of environmental contamination. Environ Health Perspect 102(9):780-781.

Burger J, Carruth-Hinchey C, Ondroff J, McMahon M, Gibbons JW, Gochfield M. 1998. Effects of lead on behaviour, growth and survival of hatchling slider turtles. J Toxicol Environ Health, A 55:495-502.

Day RD, Segars AL, Arendt MD, Lee AM, Peden-Adams MM. 2007. Relationship of blood mercury levels to health parameters in the loggerhead sea turtle (*Caretta caretta*). Environ Health Perspect 115(10):1421-1428.

Keller JM, Kucklick JR, Stamper MA, Harms CA, McClellan-Green PD. 2004. Associations between organochlorine contaminant concentrations and clinical health parameters in loggerhead sea turtles from North Carolina, USA. Environ Health Perspect 112(10):1074-1079.

Keller JM, McClellan-Green PD, Kucklick JR, Keil DE, Peden-Adams MM. 2006a. Effects of organochlorine contaminants on loggerhead sea turtle immunity: Comparison of a correlative field study and in vitro exposure experiments. Environ Health Perspect 114(1):70-76.

Keller JM, Peden-Adams MM, Aguirre AA. 2006b. Immunotoxicology and implications for reptilian health. In: Toxicology of Reptiles (Gardner SC, Oberborster E, eds). Boca Raton:CRC Press, 199-240.

Lam JCW, Tanabe S, Chan SKF, Lam MHW, Martin M, Lam PKS. 2006. Levels of trace elements in green turtle eggs collected from Hong Kong: Evidence of risks due to selenium and nickel. Environ Pollut 144(3):790-801.

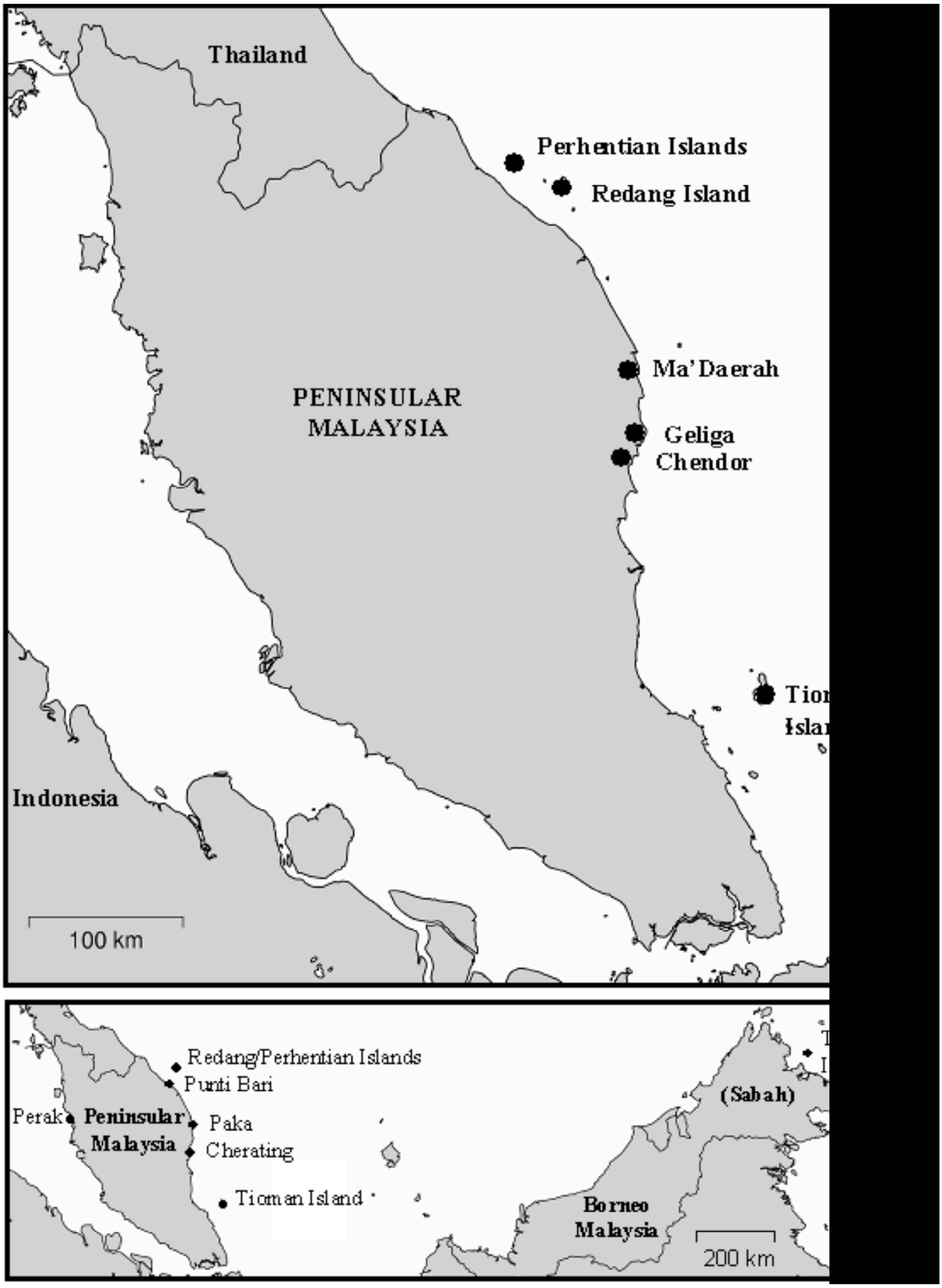
Leach RMJ, Wang KWL, Baker DE. 1979. Cadmium and the food chain: The effect of dietary cadmium on tissue composition in chicks and laying hens. J Nutr 109(3):437-443.

Podreka S, Georges A, Maher B, Limpus CJ. 1998. The environmental contaminant DDE fails to influence the outcome of sexual differentiation in the marine turtle *Chelonia mydas*. Environ Health Perspect 106(4):185-188.

Stanley TRJ, Spann JW, Smith GJ, Rosscoe R. 1994. Main and interactive effects of arsenic and selenium on mallard reproduction and duckling growth and survival. Arch Environ Contam Toxicol 26:444-451.

Vodela JK, Lenz SD, Renden JA, McElhenney WH, Kemppainen BW. 1997. Drinking water contaminants (arsenic, cadmium, lead, benzene and trichloroethylene): Effects on reproductive performance, egg quality, and embryo toxicity in broiler breeders. Poult Sci 76(11):1493-1500.

Willingham E, Crews D. 1999. Sex reversal effects of environmentally relevant xenobiotic concentrations on the red-eared slider turtle, a species with temperature-dependent sex determination. Gen Comp Endocrinol 113:429-435.


Thailand
Perhentian Islands
Redang Island
Ma'Daerah
PENINSULAR
MALAYSIA
Geliga
Chendor
Indonesia
100 km
Redang/Perhentian Islands
Punti Bari
Perak
Peninsular
Malaysia
Paka
Cherating
Tioman Island
(Sabah)
Borneo
Malaysia
200 km